Laporan Hasil Penelitian

THE EFFECTIVENESS OF *SCAEVOLA TACCADA* EXTRACT (*GAERTN ROXB*) ON THE LEVEL OF CYTOKINE IL-10 OF STRAIN *SPRAGUE DAWLEY* (FEMALE LABORATORY RATS) INDUCED BY THE *STAPHYLOCOCCUS AUREUS* BACTERIA

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ABSTRACT

Staphylococcus aureus is a bacteria causing mastitis on women, generally found after delivery and during breastfeeding and it stimulates the cytokines released. IL-10 is an anti-inflammatory cytokine that impedes cell damage on the body, so it is required to do a complementary therapy stemming from the extract of *Scaevola taccada* extract (*Gaertn roxb*). This study aimed is to examine the effectiveness of *Scaevola taccada* extract (*Gaertn roxb*) on the level of cytokine IL-10 of Strain Sprague dawley (Female Laboratory Rat) induced by the Staphylococcus Aureus Bacteria. This true experimental study employing pre and post-test control design used 18 Sprague dawley, laboratory rats, weighed between 200-250g, and divided into three groups, each of which comprised of six rats. The negative control group, positive control group and experimental group were respectively given 1ml of aqua pro injection/250grBB rats, 9.6mg/ml of antibiotic amoxicillin/250gramBB rats, and 9.6 mg of antibiotic amoxicillin/250grBB + 400mg/ml of *Scaevola taccada* extract (*Gaertn roxb*)/kgBB rats for five days. The sea lettuce plants were taken from the district of Pinrang, South Sulawesi. All groups were induced by *Staphylococcus aureus* 0.2 ml x10^8 ml/CFU. The level of IL-10 was investigated using R&D method system ELISA Rat, in which the data were analyzed using ANOVA + post hoc test formula with the significant value $\rho \leq 0.05$ and CI 95%. The empirical evidence showed that there was no significant different of cytokine IL-10 level, either before or after the induction of the bacteria *Staphylococcus aureus* for the length of ± 24 hours of all groups ($\rho > 0.05$). There was a different level of IL-10 after the treatment between all of the groups given, with ρ value < 0.05. The increase of IL-10 level was higher in the experimental group (mean ± 72,8 pg/ml) compared to the positive control group (mean ± 41, pg/ml). This research concluded that giving the extract of *Scaevola taccada* (*Gaertn roxb*) was effective in the boost of anti-inflammation cytokine IL-10, and plays central role in immune responses stimulation and the prevention of lichen imunopathology development.

Keywords : IL-10, extract of *Scaevola taccada*.

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ABSTRAK

Staphylococcus aureus adalah bakteri yang menyebabkan mastitis pada wanita, umumnya ditemukan setelah melahirkan, selama menyusui dan merangsang pelepasan sitokin. IL-10 adalah sitokin anti-inflamasi yang menghambat kerusakan sel pada tubuh, sehingga diperlukan sebagai terapi tambahan. Penelitian ini bertujuan menguji efektivitas ekstrak taccada Scaevola (*Gaertn roxb*) pada tingkat sitokin IL-10 Strain Sprague dawley (Tikus Laboratorium Wanita) yang diinduksi oleh Staphylococcus aureus. Studi eksperimental ini menggunakan desain kontrol pra dan pasca tes menggunakan 18 tikus Sprague dawley dengan berat antara 200-250g, dan dibagi menjadi tiga kelompok, yang masing-masing terdiri dari enam tikus. Kelompok kontrol negatif, kelompok kontrol positif dan kelompok eksperimen masing-masing diberikan 1 ml tikus aqua pro injeksi / 250grBB, 9,6 mg / ml tikus amoksisilin / 250gramBB antibiotik, dan 9,6 mg amoxicillin antibiotik / 250grBB + 400mg / ml ekstrak taccada Scaevola (*Gaertn roxb*) / kgBB tikus selama lima hari. Tanaman selada laut diambil dari Kabupaten Pinrang, Sulawesi Selatan. Semua kelompok diinduksi oleh Staphylococcus aureus 0,2 ml x108 ml / CFU. Tingkat IL-10 diselidiki menggunakan metode R & D sistem ELISA Rat, di mana data dianalisis menggunakan ANOVA + formula pengujian post hoc dengan nilai signifikan $\rho \leq 0,05$ dan CI 95%. Bukti empiris menunjukkan bahwa tidak ada perbedaan signifikan dari tingkat IL-10 sitokin, baik sebelum atau setelah induksi bakteri Staphylococcus aureus untuk panjang ± 24 jam dari semua kelompok ($\rho > 0,05$). Terdapat kadar IL-10 yang berbeda setelah perawatan antara semua kelompok yang diberikan, dengan nilai $\rho < 0,05$. Peningkatan kadar IL-10 lebih tinggi pada kelompok eksperimen (rata-rata ± 72,8 pg / ml) dibandingkan dengan kelompok kontrol positif (rata-rata ± 41, pg / ml). Penelitian ini menyimpulkan bahwa pemberian ekstrak *Scaevola taccada* (Gaertn) Roxb) efektif dalam meningkatkan sitokin anti-inflamasi IL-10, dan memainkan peran sentral dalam stimulasi respon imun dan pencegahan pengembangan imunopatologi lumut.

Kata kunci : IL-10, extract of *Scaevola taccada*.

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INTRODUCTION

Staphylococcus aureus is a family of *micrococcaceae*. During the microscopic investigation, the organism appeared as a group of cocci in gram positive. This bacterium has wall cells, which contain 50% of peptidoglycan, and which possess activities, such as endotoxin, that stimulate the release of sitokin generated by microphage, complementary activation, and thrombocyte aggregation. The toxin yielded by the s*taphylococcus aureus* can cause the formation of pore and induce the proinflmation that stimulates the inflammation responses (Michal B,*et.al*., 2010; Franklin D, 2016; & Sanjay K, 2017).

Staphylococcus aureus bacteria can bring about various illneses, one of which is mastitis, an inflammation occurring around the breasts, either in both or one of the breasts. The inflammation is usually accompanied either by the infection. This illness generally occurs during the period of fourty days after delivery and that of lactation; hence, it is called lactic mastitis or *peurperalis.* Mastitis may happen to everyone in any circumtances. It has been recorded that around 33% of mastitis cases were found on breastfeeding women,

occurring two weeks after the childbirth. It is also reported that approximately one out of ten (8.0%) women have experienced similar illness one month after the delivery. The cases of mastitis caused by *staphylococcus aureus bacteria* during the period of breastfeeding are found to be approximately 59%, while around 20% in week one and 26% between week two and four are not reported during which the *staphylococcus aureus* bacteria are found. In Indonesia is projected around 3-20% occurance of mastitis on breastfeeding women every year (WHO, 2000; Kamal K *et al*., 2012; Tri A dan Sumarni, 2014; Pilar M *et.al*, 2014; Alecssandra et.al, 2015; Vishnu K, *et al* 2015; Mahnaz Z *et al*., 2017 & Meabh C, *et al*., 2015).

Provided the response of the immune is active, cell T-helper detects the antigen microba. Several of the cells T-helper are cultivated together with the prime cells of the antigen microba, that is the *Antigen Precenting Cell* (APC) and monocyte, to discover the factors of exogoneous polarization. The sitokin is subsequently released by each of innate receptor due to the presence of pathogenic bacteria (Christina E. Zielinski, 2014).

Interleukin-10 is the inhibitor of macrophage and dendritic cells that play role in controlling the reaction of the non-specific immune and immune celluler. IL-10 is produced mainly by the activated macrophage. Sitikon IL-10 is used as an anti-inflammation lowering the action or production from one or more pro-inflammatory sitoksin of proteins generated by nerves, neuron, *neuron, glia cells, endotel cells, fibroblast cells*, muscles, immune cells , or other types of cells. Sitokin anti-hipernosisepsis IL-10 is produced by various types of cells, such as *limfosit, monosit, macrophage, and mast cells* (Kumar A, *et al*, 2000; Iyer S, *et al*; 2013; & Baratawidjaja K.*et al*, 2014).

The use of medicinal herbs nowadays has yielded oceans of benefits that are used both as the primary and complementary therapy to escalate one's immune. One of these herbs is the sea lettuce (*scaevola taccada*), which can be maded as traditional medicines. It benefits after-birth women as a cleansing. Its roots are used to treat stomache, and its barks and leaves can be used to prevent a relapse. Juice made of its barks and roots are respectively used to cure ringworm and beriberi, , and its roots are used to Jus dari kulit kayu digunakan untuk mengobati kurap, akar digunakan untuk mengobati beri-beri, syphilis, and dysentery. The leaves can also be made use as a cure for digestive system, carminative, antitumor, antiinflammation, cough, and tuberculosis. A study unveiled that former phytochemical screening of *Scaevola taccada* leaves extract contains alkaloid, flavonoid, lipid, terpenoid, glikosida and saponin that can used ad antiinflammation (Irene J, *et.al*., 2007; Chandran A, Arunachalam G; 2013a, 2015b, Rahmawati, *et.al.,* 2014; Suthiwong J *et.al.* 2016; Amran N, 2017 & Sutar N, *et.al*., 2017).

Some empirical evidence show that the relatively increased cases of mastitis is a health problem that may harm women's health, either after birth or during the breastfeeding period, because of the presence of the inflammatory reaction, which yields inflammation, not to mention an abscess on the breasts that requires a therapy or a medical treatment. To tackle such problems, sea lettuce can be used as traditional medicinal herbs. Therefore, the researchers proposed this study examining the effectiveness of *Scaevola taccada* extract (*Gaertn roxb*) on the level of Sitokin Il-10 of Strain Strain Sprague Dawly

(Female Laboratory Rat) induced by the Staphylococcus Aureus Bacteria.

METHOD

This true experimental research is a laboratory research with pre- and post-test control design. The study was conducted at the laboratory room of the hospital of University of Hasanudin (Unhas) to examine elisa and the thrive of *s. aureus* bacteria. Biofarmaka Lab was used to dry the plants; the farmacy building of the state Islamic University of Makassar (UIN) was for the extraction and phytochemical tests; and the animal Lab of Unhas was used to do the adaptation process for the rats til the end of the treatment.

The sample in this study was eighteen Strain *Sprangue Dawley* rats weighing between 200-250g, which were divided into three groups, each of which comprised of six rats. The negative control group, positive control group and expiremental group were respectively given 1ml of aqua pro injection/250grBB rats, 9.6mg/ml of antibiotic amoxicillin/250gramBB rats, and 9.6mg of antibiotic amoxicillin/250grBB + 400mg/ml of *Scaevola taccada* extract (*Gaertn roxb*)/kgBB rats for five days. The sample was conducted based on the *research guidelines for evaluating the savety and efficaty of herbal medicine* in accordance with World Health Organization (WHO).

The instruments employed in this study were the sea lettuce collected along the seashore of Suppa district, Pinrang regency, South Sulawesi, and extracted at the Unhas laboratory of Biofarmaka. The rats, Strain Sprague Dawly, were tested weighing between 200-250g and treated with animal feed, a*qua pro injection, 5 pieces of a*ntibiotic amoxicillin @500mg, *staphylococcus aureus bacteria standard taken from* the Lab of Unhas hospital breeded by Mc.Farland 2 x 10^8ml/CFU. The equipments used were a laboratory animal housing, digital scale, Elisa kit R&D, handgloves, micropipet, and 1mL spoit. Histopathology test was done to figure out the spread of inflamed cells. The current study gained etical permission and recommendation on the date of 18th December 2017 with the decree no. 1077/H4.8.4.5.31/PP36-KOMETIK/2017.
The data were computed and analysed using computers. The effect of giving sea lettuce extracts on the level of sitokin IL-10 was presented in terms of mean score (standard deviation) with interval confidence (95% CI). Bivariate test using Anova along with Post hoc test was run at the significant value $\rho \leq 0.05$.\

RESULTS

Table 1. Phytochemical test results of sea lettuce's chemical substances (*Scaevola taccada* (gaertn)Roxb.

Name of Sample	Compound identification categories						
	Alkaloid			Flavonoid	Steroid/ tripernoid	Saponin	Tanin
	Drag	LB	Mayer				
Sea lettuce extracts (Scaevola taccada(Gaertn)Roxb)	+	+	+	+	+	+	+

Note: + (positive): There was an indication of bioactive compound.

Table 2. Average weigh of rats in each of the group

Weigh	Mean ± SD	Min-Max	ρ value
Negative control group	226 ± 12	213 - 239	0.325
Positive control group	220 ± 12	208 - 233	
Expiremental group	230 ± 10	221 - 240	

**One way ANOVA.* Significant value ρ ≤ 0.05. There was no difference of weigh rats in all groups, either in the negative, positive, and expiremental group (ρ = 0.325).

Table 3. The average difference of IL – 10 level in each of female rat, strain *Sprague Dawly*, before and after the induction of *Staphylococcus aureus* bacteria, and after the treatment.

Groups	Level of IL-10 (pg/ml)			ρ value
	Before Induction	After Induction S.A	After *treatment*	
	Mean ± SD	*Mean* ± SD	*Mean* ± SD	
Negative control (n =6)	111.2 ± 10.5	34.5 ± 8.2	24.1 ± 5.6	**0.00[a]**
Positive control (Antibiotic *amoxicillin* 9.6 mg/250grBB) (n =6)	115.1 ± 16.9	33.7 ± 8.2	75.4 ± 7.6	**0.001[a]**
Treatment (Antibiotic *amoxicillin* 9.6 mg/250grBB + sea lettuce extracts 400 mg/kgBB (n =6)	120.4 ± 16.7	30.8 ± 6.6	103.6 ± 11.5	**0.000[a]**
Value ρ	**0.58[b]**	**0.68[b]**	**0.000[b]**	

ANOVA ([a]repetead ANOVA) significant value ρ ≤ 0,05. There was a difference in the level of sitokin IL-10 in the female rats, strain Sprague Dawly of all groups, either before, after the induction of S.aureus and after the treatment (ρ ≤ 0,05). [b]One Way-ANOVA operated in the previous measurement and after the induction of s.aureus in each of the group showed no difference in the level of IL-10 either bofre and after inducing the s.aureus (ρ > 0,05). There was a difference in the level of IL-10 after the treatment in each of the group (ρ < 0,05).

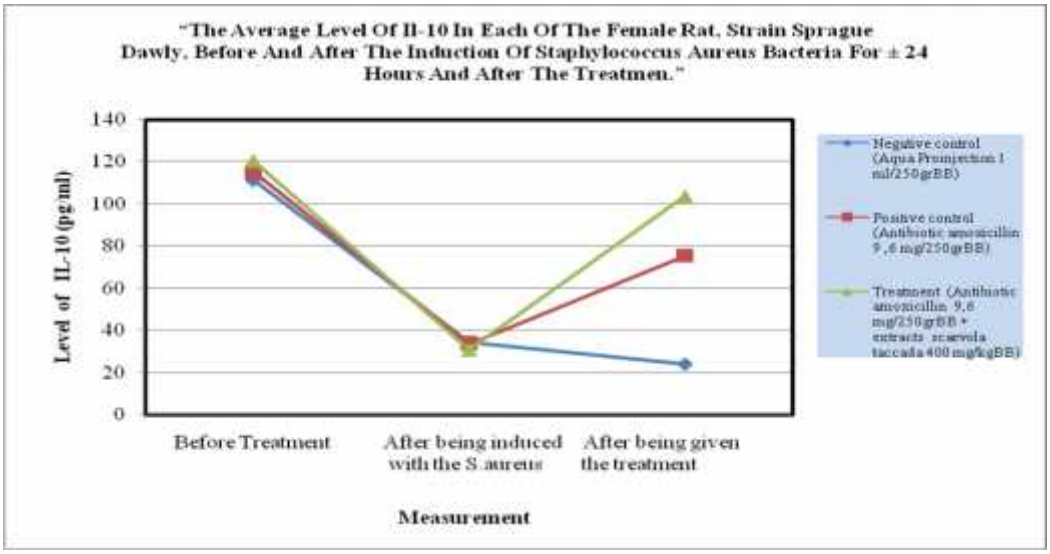


Figure 1. Trend of the level of IL-10 in each of the female rat, strain Sprague Dawly, before, after being induced with the S.aureus and after being given the treatment.

Note: the level of IL-10 in each of the group before the induction of the s. aureus bacteria was not significantly different, and after the induction of the bacteria, the level of IL-10 dwindled. After given the treatment, that of the positive control group and treatment groups was increasing, while that of negative control group was decreasing.

Table 4.5. The analysis of the difference of IL-10 level before, after the induction of *s. aureus* and after given the treatment in each of the group.

Measurement	Mean ± SD	Mean difference	Value ρ*	Value ρ
Before the *treatment*				
Control Negative	111.2 ± 10.5	-3.8	1.00	0.58
Control Positive	115.1 ± 16.9			
Control Negative	111.2 ± 10.5	-9.1	0.92	
Treatment	120.4 ± 16.7			
Control Positive	115.1 ± 16.9	-5.3	1.00	
Treatment	120.4 ± 16.7			
After the induction of *s. aureus* bacteria				
Control Negative	34.5 ± 8.2	0.9	1.00	0.68
Control Positive	33.7 ± 8.2			
Control Negative	34.5 ± 8.2	3.8	1.00	
Treatment	30.8 ± 6.6			
Control Positive	33.7 ± 8.2	2.9	1.00	
Treatment	30.8 ± 6.6			
After the *treatment*				0.00
Control Negative	24.1 ± 5.6	-51.3	0.00	
Control Positive	75.4 ± 7.6			
Control Negative	24.1 ± 5.6	-79.4	0.00	
Treatment	103.6 ± 11.5			
Control Positive	75.4 ± 7.6	- 28.0	0.00	
Treatment	103.6 ± 11.5			

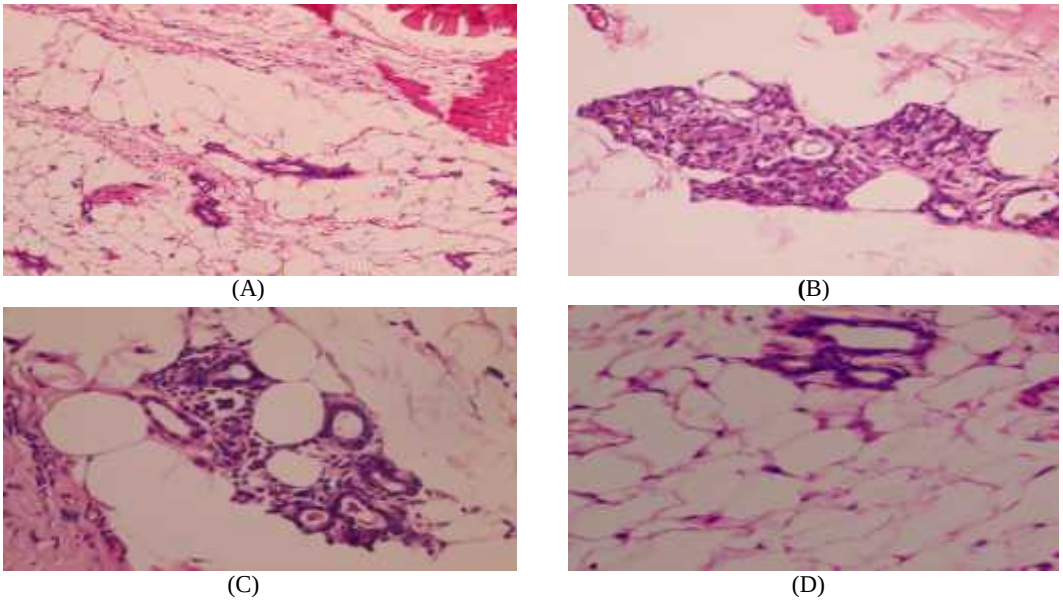
One Way ANOVA + post hoc Bonferroni, at the significant value ρ ≤ 0,05. There was no difference in the level of sitokin IL-10, either before or after the induction of *staphylococcus aureus* bacteria for ± 24 hours in each of the group. There was a difference in the level of IL-10 after the treatment between the groups.

Table 4.6 . analysis of the different level of IL-10 between the female strain *Sprague dawly* before, after the induction of *Staphylococcus aureus* bacteria and after the treatment.

Measurement	Mean ± SD	Difference of Mean	Value ρ*	Value ρ
Control Negative				
Before	111.2 ± 10.5	76.6	0.000	0.000
After the induction of *s.aureus*	34.5 ± 8.2			
Before	111.2 ± 10.5	87.1	0.000	
After the treatment	24.1 ± 5.6			
After the induction of *s.aureus*	34.5 ± 8.2	10.4	0.049	
After treatment	24.1 ± 5.6			
Control Positive				
Before	115.1 ± 16.9	81.4	0.000	0.001
After the induction of *s.aureus*	33.7 ± 8.2			
Before	115.1 ± 16.9	39.6	0.008	
After the treatment	75.4 ± 7.6			
After the induction of *s.aureus*	33.7 ± 8.2	-41.8	0.000	
After treatment	75.4 ± 7.6			
Perlakuan				
Before	120.4 ± 16.7	89.6	0.000	

After the induction of *s.aureus*	30.8 ± 6.6			0.000
Before	120.4 ± 16.7	16.8	0.43	
After the treatment	103.6 ± 11.5			
After the induction of *s.aureus*	30.8 ± 6.6	-72.7	0.000	
After treatment	103.6 ± 11.5			

*Repeated ANOVA + post hoc Bonferroni, at the siginifcant value $\rho \leq 0{,}05$. There was a difference in the level of sitokin IL-10 in the Control negative and positive groups, either before, after the induction of s. aureus for ± 24 hours and after the treatment. There was no difference in the level of sitokin IL-10 before and after given the treatment in the treatment group.



(A) (B) (C) (D)

Figure 2. Histopathology test results

Note: (A) it is the microscopic picture of the rats' breast in the control normal group showing the blood vessels, Normal epithelial cells are surrounded by connective tissue and the presence of lactiferous ducts. (B) It is the microscopic picture of the rat's breast in the control negative group showing the inflammation of around 120 cells PNM around the connective tissue and epithelial cell wall. (C) It is the microscopic picture of the rat's breast in the control positive group presenting the inflammation of about 80 cells PNM around the connective tissue and epithelial cell wall. (D) It is the microscopic picture of the rat's breast in the control positive group presenting the inflammation of about 30 cells PNM around the connective tissue and epithelial cell wall.

DISCUSSION

The results of the analysis showed that there was no difference in the level of sitokin IL-10 after the induction of *Staphylococcus aureus* bacteria within about 24 hours in each of the group. The level of IL-10 in the female *Sprangue dawly* after the induction of the *Staphylococcus aureus* bacteria for about 24 hours experienced a drop with average value of decrease in the control negative group (76.7 pg/ml), control positive group (81.4 pg/ml) and treatment group (89,6 pg/ml). The results of histopathological tests showed clear inflammation of 120 cells PNM surrounding the *ductus lactiferus*, epithelial cell wall and connective tissue. This indicates that after the induction of the *Staphylococcus aureus* bacteria for about 24 hours, all of the groups were affected by the bacteria. Therefore, all the rats could be ascertained to have got mastitis.

In this study was unveiled the difference between each group after the treatment. In the control negative group, the level of IL-10 dwidled after the treatment (*aqua pro-injection* 1 ml/200grBB) with average drop

of around ± 10.4 pg/ml. The level of sitokin IL-1o in the control positive group escalated after given the antibiotic amoxicillin 9.6 mg/250gramBB for five days with average increase around ± 41.pg/ml. On the other hand, in the treatment group was an increase of the level of sitokin IL-10 after given the treatment, antibiotic amoxicillin 9.6 mg/250grammBB + sea lettuce extracts 400mg/kgBB for five days with average increase around ± 72.8 pg/ml. This shows that the treatment group has higher increase in the level of IL-10 compared to control positive group.

The findings accounted for previous research findings, in which the level of IL-10 experienced deficiency after being affected by the *Staphylococcus aureus* bacteria for about ± 24 hours. The number of bacteria will continuously increase, and between 72 hours (3 days) and ± 168 hours (7 days), it will continuously drop till the zero point within about ± 168 hours(7 days) or even cannot be detected (Gjertsson et.al, 2002; John L, 2017).

The mastitis caused by the *Staphylococcus aureus* bacteria stimulates the inflammatory responses indicated by the provision of inflamed cells around the epithelial celss and breast tissue. Sitokin IL-10 as an antiinflammation will defend the the host primarily the epithelial cells and breast tissue to reduce the cells damage, prevent the inflammatory activity and responses the immune system. Sitokin IL-10 plays central roles in hampering the the responses of immune against pathogen and secure normal homeostasis (Kumar A, *et al,* 2000; Wenjun O, et al, 2011, G. Andres Contreras & Juan M, 2011; Iyer S, *et al*; 2013).

The role of IL-10 is to manage the function of a large number of different immune cells, reduce the production of inflammatory mediator, and prevent the presence of antigen. In the mechanism of mastitis secretion, various toxic substances overwhelmedly generated by *S.aureus* will destroy the normal cells as a result of the produced *Reactive Oxygen Species* (ROS) and enzyme lysozyme produced by neutrophil and macrophage. Such a condition stimulates more inflammation indicated by increase of pro-inflammatory production, which subsequently reduces the level of IL-10 in body automatically, and the production of pro-inflammatory sitokin will activate more immune cells heading to the infection area, which damages the blood vessels walls as well as the organ disfunction. (Fanny N et al, 2015; Kumar A, 2000; Sabat R, 2010; Abbas AK, *et al*, 2012; Baratawidjaja K.*et al*, 2014; Wendy V, 2014; Manzanillo Paolo, *et al* ,2015; Yagmur Y, 2016).

Giving antibiotic for treating mastitis can be a quick resolution. One of the antibiotics is *amoxicillin*. The research findings showed that given on antibiotic, amoxicillin, does not elevate the level of IL-10 significantly; hence, the patient needs an complementary therapy (WHO, 2000; Demartini et al, 2004; The Women, 2012; Kamal, 2012; Alasiry E, 2013; Jahanfar et al; 2013).

Sea lettuce has bioactive substances, which include flavonoid, saponin, alkaloid, dan tannin, that can be anti-inflammations. Flavonoid working on *endothelium mikrovaskular* to lower the occurrence of hipermeability and inflammation. Several compounds of flavonoid can deter the release of arachidonic acid and secretion of lysozom enzyme from membrane by blocking the current of Cyclooxygenase and *lipoksigenase* which lessen the level of prostaglandin and leukotriene (inflammatory mediator). The saponins and alkaloids contained in sea lettuce inhibit the release of pro-inflammatory mediators and

release of TNF-α, IL-1β, IL-6 cytokines from LPS monocytic cells (Macrophage and Th1). On the other hand, the role of steroids as an anti-inflammatory agent will inhibits various cells producing important factors to generate inflammatory cells. The findings above accounted for the other empirical evidence which show that steroids are able to regulate and improve the production of IL-10 selectively by igniting the activity signals in the monocyte cells (Mozo L & Guiterez, 2004; Jean P et. al, 2006; Chattopadhyay; 2012).

The increase of IL-10 can prevent the development of immunopathology occurring due to the response of protective immune against the acute and chronic infection. Sitokin IL-10 is essential in curing the integrity and homeostasis of epithel cell tissue and in facilitating the process of curing wounds, infection, or abscess (Jean L et al, 2006; Wenjun O, et al, 2011).

The researcher assume that sea lettuce extracts can be a complementary therapy in treating mastitis becaseu of its ability to promote the level of IL-10, which plays roles as an anti-inflammatory; hence, it prevents the reaction of inflammation caused by microogranism. Further studies are needed to measure the level of quantitative tests in the extract of sea lettuce, so that other clinical tests on the patients with mastitis can be done.

CONCLUSION

This research concluded that giving the extract of *Scaevola taccada* (Gaertn)Roxb) was effective in the boost of sitokin IL-10 level as an anti inflammation that plays central role in the stimulation of immune responses and the prevention of lichen imunopatologia development. The extract of sea lettuce (*Scaevola taccada* (Gaertn)Roxb)) can be given as a complementary therapy.

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